

Comparative Efficacy of an Imidacloprid/Flumethrin Collar (Seresto®) and an Oral Afoxolaner Chewable (NexGard®) against Tick (*Dermacentor variabilis* and *Amblyomma americanum*) Infestations on Dogs: a Randomised Controlled Trial

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Abstract

This randomised controlled laboratory study demonstrated the residual speed of efficacy of an imidacloprid/flumethrin collar (Seresto®, Bayer) for the control of ticks (*Dermacentor variabilis*, *Amblyomma americanum*) at 6 and 12 hours post-infestation on dogs when compared to oral afoxolaner (NexGard®, Merial). Dogs were randomised by pre-treatment tick counts: Group 1) imidacloprid 10 % (w/w)/flumethrin 4.5 % (w/w) collar, 2) afoxolaner chewable (dosage 3.1–6.2 mg/kg), and 3) non-treated controls. Ticks (50/species/dog) were infested on days 3, 14, 21, and 28; live (attached and non-attached) and dead attached ticks were counted 6 and 12 hours later. Efficacy against live *D. variabilis* at 6 hours for Group 1 was 95–100 % and for Group 2 was 38–48 %; efficacy at 12 hours

for Group 1 was 97–100 % and for Group 2 was 27–59 %. Efficacy against *A. americanum* at 6 hours for Group 1 was 94–100 % and for Group 2 was <0–38 %; efficacy at 12 hours for Group 1 was 98–100 % and for Group 2 was 1–40 %. Live and total (total live and dead attached) tick counts in Group 1 against both tick species were significantly lower ($p \leq 0.05$) than Group 2 and 3 at all time points. The number of live or total ticks on Group 2 dogs was never significantly lower when compared to the respective number of ticks on Group 3 (controls). This study demonstrated that an imidacloprid/flumethrin collar was highly efficacious (94–100 %) at repelling and killing ticks on dogs at 6 and 12 hours post-infestation and was more efficacious than afoxolaner on all challenge days.

Introduction

Dermacentor variabilis and *Amblyomma americanum* belong to the Ixodidae family of ticks, also known as hard ticks due to their hard dorsal surface or scutum. The two other genera within the Ixodidae family that commonly infest dogs in North America are *Ixodes* and *Rhipicephalus* (Bowman 2009). Most species, if not all, from these genera are 3-host ticks; they feed on 3 different hosts during their life cycle (Sonenshine 2013). This feeding behavior makes ticks excellent vectors of diseases as they may acquire pathogens not only via vertical transmission from the adult female (transovarial transmission) but also while feeding on different hosts as larvae or nymphs (transstadial transmission) (Bowman 2009).

Tick-borne diseases are an emerging infectious threat with ticks acting as the leading source of pathogen transmission to animals (Chomel 2011). Ticks are capable of transmitting a myriad of pathogens, including viruses, protozoa, and bacteria. Specifically, *D. variabilis* and *A. americanum* can vector *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever, as well as many *Ehrlichia* species (Chomel 2011; Goddard and Varela-Stokes 2009). In addition, *D. variabilis* can vector *Theileria equi* and *A. americanum* can vector *Rickettsia parkeri* and *Hepatozoon americanum* in several countries throughout the world.

It was previously thought that the transmission of diseases causing organisms by ticks took over 24 hours. However, recent research evaluating *Ehrlichia canis* transmission by *Rhipicephalus sanguineus* in dogs revealed that transmission can occur as fast as 3 hours (Fourie et al. 2013b). Other Rickettsial pathogens are thought to be transmitted within 4–24 hours and transmission of *Borrelia burgdorferi*, by certain *Ixodes* species, has been documented to occur in less than 24 hours (Cook 2015; Nicholson et al. 2010). While transmission times vary depending on the pathogen involved, the faster a tick is repelled and/or killed the less likely the tick will be able to attach and

feed, thereby limiting the risk for transmission of disease causing organisms.

The imidacloprid/flumethrin collar (Seresto®, Bayer) has been marketed in the United States since 2013 with 8 month repel and kill efficacy against fleas (*Ctenocephalides felis*) and ticks (*Ixodes scapularis*, *D. variabilis*, *R. sanguineus*, and *A. americanum*), one month efficacy against chewing lice, and also aids in the control and treatment of Sarcoptic mange. Recently, the oral chewable afoxolaner (NexGard®, Merial) was marketed with one month kill efficacy against fleas (*C. felis*) and ticks (*R. sanguineus*, *D. variabilis*, *I. scapularis*, and *A. americanum*). This randomised controlled comparative laboratory study was designed to demonstrate the residual speed of efficacy of Seresto® for the control of ticks (*D. variabilis* and *A. americanum*) at 6 and 12 hours post-infestation on dogs when compared to 2 competitive products and a non-treated negative control. Results from 3 groups are reported here.

Materials and methods

Animals

Dogs were included if they were over 6 months of age, determined to be healthy based on physical examination, not pregnant, were able to harbor an adequate tick infestation, and were not exposed to a previous insecticide/acaricide within 90 days of study onset. Initially, 48 dogs were evaluated for inclusion in the study. The dogs were evaluated for the ability to harbor adequate tick infestations. Briefly, 48 dogs were each infested with approximately 50 *D. variabilis* on study day –7. On study day –5, tick counts were performed to remove all live ticks. Dogs were then ranked by pre-study live tick counts in descending order and randomised in sets of 4, excluding 8 dogs with the lowest tick counts. The results from 3 groups of 10 animals, Group 1) Seresto®, Group 2) NexGard®, and Group 3) non-treated controls, are reported here. The fourth group, fluralaner (Bravecto®, Merck),

along with dogs in Groups 1 and 3 are presented in a separate manuscript.

Products

Both products were administered in accordance with the manufacture's guidelines and according to the weight of each dog prior to treatment. On study day 0, Group 1 dogs had Seresto® collars containing imidacloprid 10% (w/w)/flumethrin 4.5% (w/w) applied around the neck. The length was adapted to fit the dog according to label directions with the extra length cut off and secured with a ratchet mechanism. Collars were applied according to the dog's bodyweight (collar dose ranges: small collar <18lbs, large collar >18lbs). Group 2 dogs were treated orally with NexGard® (afoxolaner) at a dosage of 3.1–6.2 mg/kg. Two size chews were available and dosed based on body weight: 28.3 mg and 68 mg. The control group remained non-treated.

Experimental infestations

Dogs were infested with *D. variabilis* and *A. americanum* based on a predetermined schedule. Each infestation consisted of approximately 50 ticks of each species applied along the dog's dorsal midline from shoulders to hips.

Experimental design

This laboratory study was conducted in accordance with VICH GL9 Good Clinical Practices (GCP), June 2000 (FDA Guidance for Industry 85, May 2001) and applicable standard operating procedures. Dogs were individually housed in runs for the duration of the study. They were bathed with a mild, non-medicated shampoo, thoroughly combed, and allowed to acclimate for 11 days prior to the initiation of treatments. Concomitant treatments were prohibited, except where deemed necessary and would not influence the performance of any product.

Following product administration, each dog was infested with 50 *D. variabilis* and 50 *A. americanum* on study days 3, 14, 21, and 28. Tick counts were performed at 6 hours (thumb count) and 12 hours

(full body comb count with tick removal) following infestation to assess the speed of repellency and/or kill; all ticks on the dogs were counted including live (attached and non-attached) and dead attached ticks.

Clinical monitoring

The dogs were observed at least once daily during the acclimation period and a physical examination was performed on each dog prior to starting the study to verify the health status. For the remainder of the study, dogs were observed daily until study completion, at which time dogs were returned to the supplier. On study day 0, dogs were also observed 2 and 4 hours after product administration for adverse events.

Efficacy determination

Total live (attached and non-attached) and dead attached tick counts were determined and recorded. Individual live tick counts were used to calculate a geometric mean (GM) for each group at each time point on the specified study days. For each post-treatment tick count, efficacy was calculated using Abbott's Formula. Percent efficacy (% reduction) was determined by comparing the GM number of live ticks retained on the treated group to the GM number of live ticks retained on the non-treated negative control group using the following formula:

$$\% \text{ efficacy} = \frac{\text{GM tick count control} - \text{GM tick count (treatment)}}{\text{GM tick count (control)}}$$

Data analysis

For *D. variabilis*, the assumption of equally distributed tick-ridding ability was assessed by descriptively summarising the pre-study tick counts. The statistical method comparing post-treatment tick counts utilised the pre-study tick counts for each animal. The efficacy of the treated groups, relative to the control group, was computed with Abbott's formula. Arithmetic mean counts and geometric means were both used in the efficacy calculation.

Table 1 *Dermacentor variabilis* efficacy and geometric mean live (attached and non-attached), dead attached, and total (total live and dead attached) tick counts after treatment with Seresto® or NexGard®

Study Day	Day 3		Day 14		Day 21		Day28	
Hours post-infestation	6h	12h	6h	12h	6h	12h	6h	12h
Seresto®								
Mean # live	1.5 ^b	1.0 ^b	0.0 ^b	0.0 ^b	0.2 ^b	0.0 ^b	0.1 ^b	0.1 ^b
Mean # dead attached	0.1 ^a	1.1 ^{ab}	0.8 ^a	0.5 ^a	0.3 ^a	0.5 ^a	0.2 ^a	0.2 ^a
Mean # total	1.6 ^b	2.1 ^b	0.8 ^b	0.5 ^b	0.4 ^b	0.5 ^b	0.2 ^b	0.2 ^b
% Efficacy ^c	94.9	97.0	100	100	99.0	100	99.7	99.8
NexGard®								
Mean # live	16.0 ^a	14.1 ^a	11.6 ^a	17.9 ^a	10.2 ^a	17.3 ^a	12.9 ^a	21.6 ^a
Mean # dead attached	0.4 ^a	2.7 ^b	0.3 ^a	1.8 ^a	0.4 ^a	1.1 ^a	0.0 ^a	0.4 ^a
Mean # total	16.6 ^a	20.2 ^a	11.9 ^a	21.4 ^a	10.5 ^a	19.2 ^a	12.9 ^a	22.0 ^a
% Efficacy ^c	43.7	59.3	38.5	38.6	48.4	40.4	38.5	27.2
Control								
Mean # live	28.5 ^a	34.6 ^a	18.9 ^a	29.2 ^a	19.8 ^a	29.0 ^a	20.9 ^a	29.6 ^a
Mean # dead attached	0.0 ^a	0.0 ^a	0.2 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
Mean # total	28.5 ^a	34.6 ^a	19.3 ^a	29.2 ^a	19.8 ^a	29.0 ^a	20.9 ^a	29.6 ^a

^{a,b} Values down columns with unlike superscripts are significantly different ($p \leq 0.05$)

^c % Efficacy calculated using Abbott's Formula

Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

Geometric means were calculated following transformation using a logarithmic method (averaging the transformed values, and converting the average using antilog to represent a geometric mean). Because some animals might have had zero (0) tick counts, all counts were modified by adding one (1) to each prior to logarithmic transformation. Also, one (1) was subtracted from the antilog value to meaningfully represent the geometric mean for each group. Only live tick counts were used to calculate efficacy.

Live, dead and total (total live and dead attached) tick counts for each species were analysed

separately. Log (tick counts+1) were analysed with a repeated measures analysis of covariance (RMANCOVA) including terms for treatment (TRT), animal (random), study day (DAY), and the interaction of treatment and study day (TRT x DAY), using the pre-treatment tick counts as a covariate (for *D. variabilis* only, no baseline covariate when analysing for the other tick species). SAS PROC MIXED (SAS® Institute, Cary, NC) was used for analysis with the covariance structures 'AR(1)' and 'ARH(1)' for data collected on equal intervals, or 'CS' and 'CSH' for data collected on unequal intervals. Results from the

Table 2 *Amblyomma americanum* efficacy and geometric mean live (attached and non-attached), dead attached, and total (total live and dead attached) tick counts after treatment with Seresto® or NexGard®

Study Day	Day 3		Day 14		Day 21		Day28	
Hours post-infestation	6h	12h	6h	12h	6h	12h	6h	12h
Seresto®								
Mean # live	0.7 ^b	0.2 ^b	0.0 ^b	0.0 ^b	0.1 ^b	0.1 ^b	0.1 ^b	0.0 ^b
Mean # dead attached	0.2 ^a	0.7 ^{ab}	1.1 ^a	0.7 ^a	0.7 ^a	0.4 ^{ab}	0.3 ^a	0.3 ^a
Mean # total	1.0 ^b	0.9 ^b	1.1 ^b	0.7 ^b	0.7 ^b	0.4 ^b	0.4 ^b	0.3 ^b
% Efficacy ^c	94.2	98.4	100	100	99.6	99.7	99.5	100
NexGard®								
Mean # live	12.4 ^a	9.8 ^a	7.5 ^a	9.6 ^a	12.1 ^a	15.1 ^a	12.3 ^a	13.1 ^a
Mean # dead attached	0.1 ^a	2.9 ^b	0.5 ^a	1.1 ^a	0.3 ^a	2.1 ^b	0.0 ^a	1.1 ^a
Mean # total	12.6 ^a	13.9 ^a	8.1 ^a	11.0 ^a	12.7 ^a	18.7 ^a	12.3 ^a	15.0 ^a
% Efficacy ^c	-1.7	31.6	12.6	0.8	37.6	35.4	18.5	40.0
Control								
Mean # live	12.2 ^a	14.4 ^a	8.6 ^a	9.6 ^a	19.4 ^a	23.4 ^a	15.1 ^a	21.8 ^a
Mean # dead attached	0.0 ^a	0.0 ^a	0.1 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
Mean # total	12.2 ^a	14.4 ^a	8.8 ^a	9.6 ^a	19.4 ^a	23.4 ^a	15.1 ^a	21.8 ^a

^{a,b} Values down columns with unlike superscripts are significantly different ($p \leq 0.05$)

^c % Efficacy calculated using Abbott's Formula

Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

model with the smallest Akaike's Information Criterion were used.

If the interaction of treatment and study day was significant at the 0.05 level, multiple group pairwise comparisons were generated using a Bonferroni alpha adjustment for multiple group comparisons. These simple effect pairwise comparisons were obtained from the TRT x DAY interaction. If the interaction term was not significant ($p > 0.05$), the TRT main effect was evaluated. If the TRT main effect was not significant ($p > 0.05$), the results were deemed not significant and no further analyses were conducted. If the TRT main

effect was significant ($p \leq 0.05$), multiple group pairwise comparisons were generated using a Bonferroni alpha adjustment for multiple group comparisons across the pooled time points. In addition, dead attached ticks and total ticks were compared across groups. However, pre-treatment *D. variabilis* counts were not used as a covariant when dead attached or total ticks were analyzed. All four treatment groups were analysed together, however only the results from Groups 1, 2 and 3 are reported here. Software from SAS® Institute, Cary, NC version 9.3 was used for all analyses.

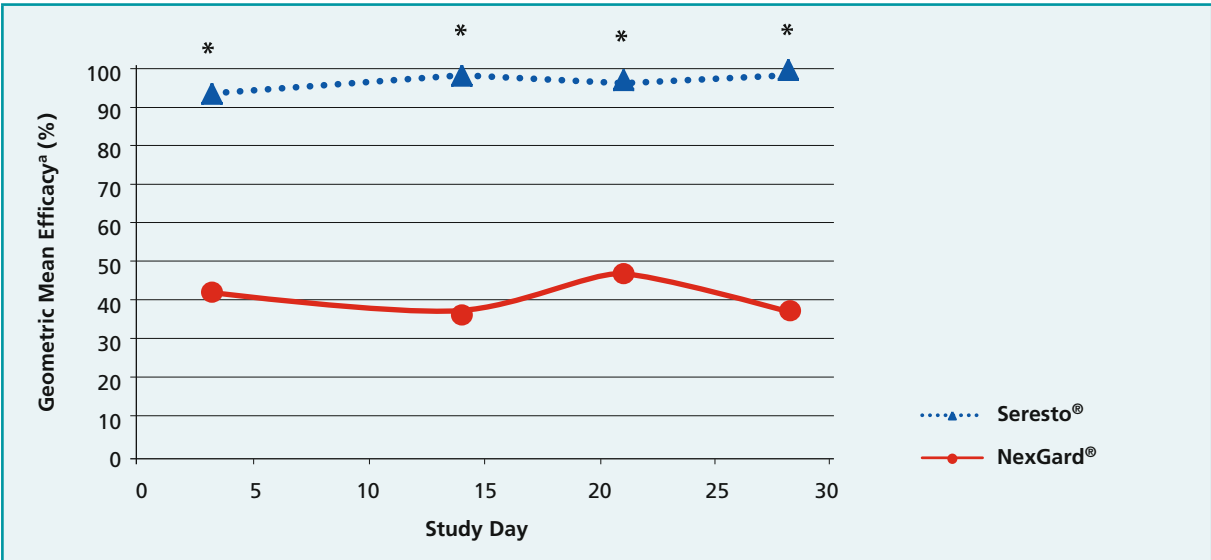


Fig. 1 Efficacy 6 hours post-infestation with *D. variabilis* after treatment with Seresto® or NexGard®
 * Live tick counts significantly different ($p \leq 0.05$) as compared NexGard® and control group
^a % Efficacy calculated using Abbott's Formula

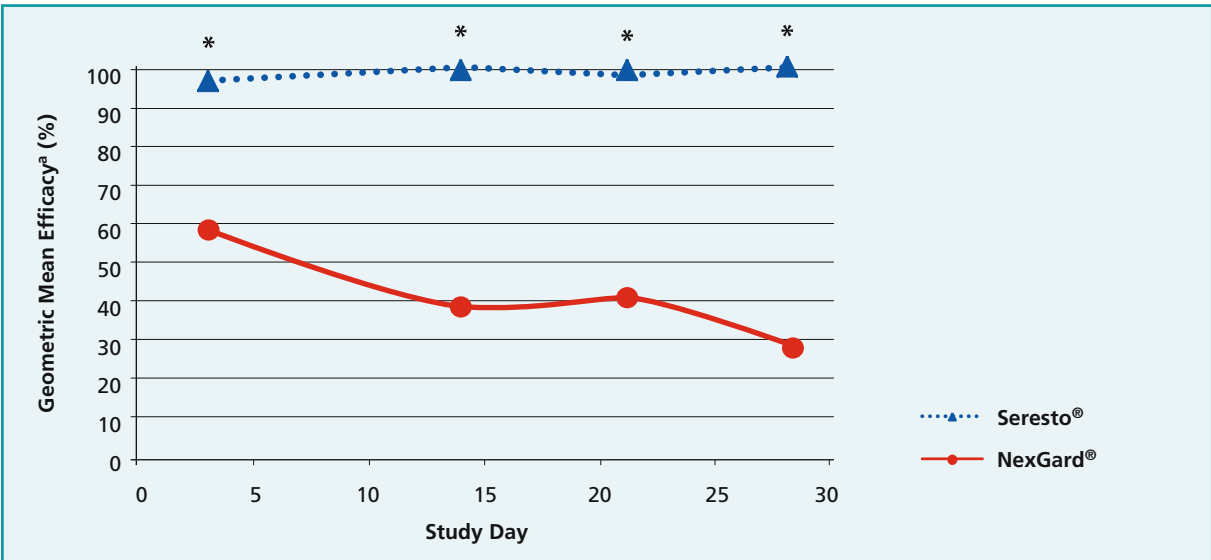
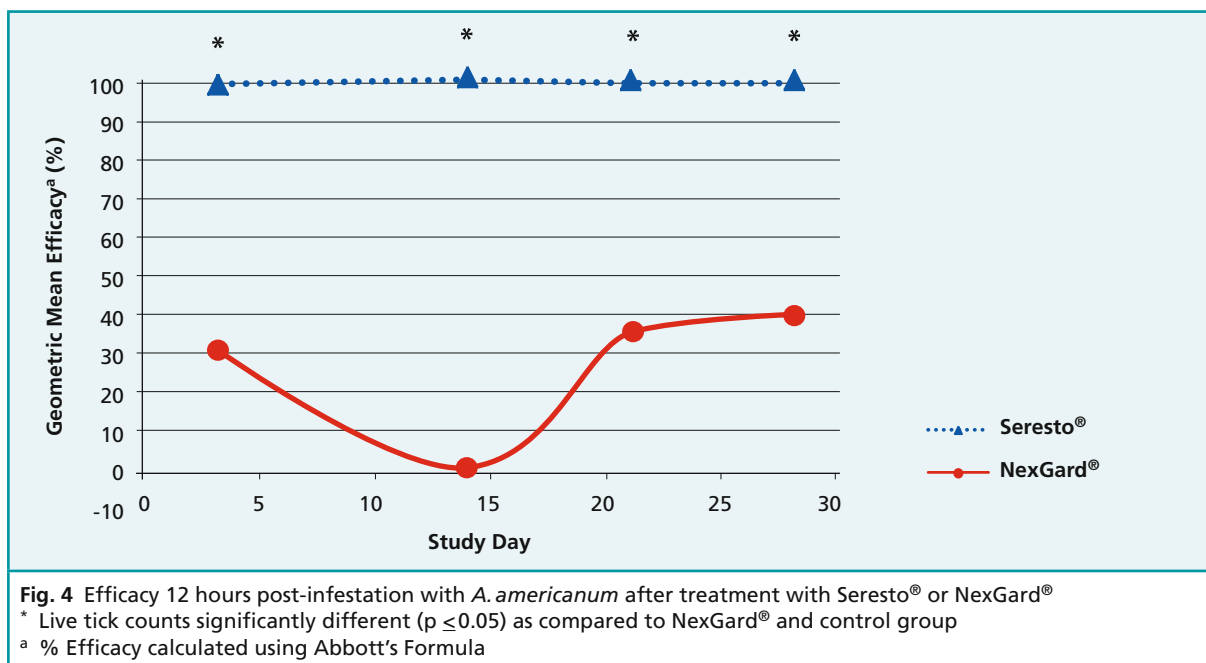
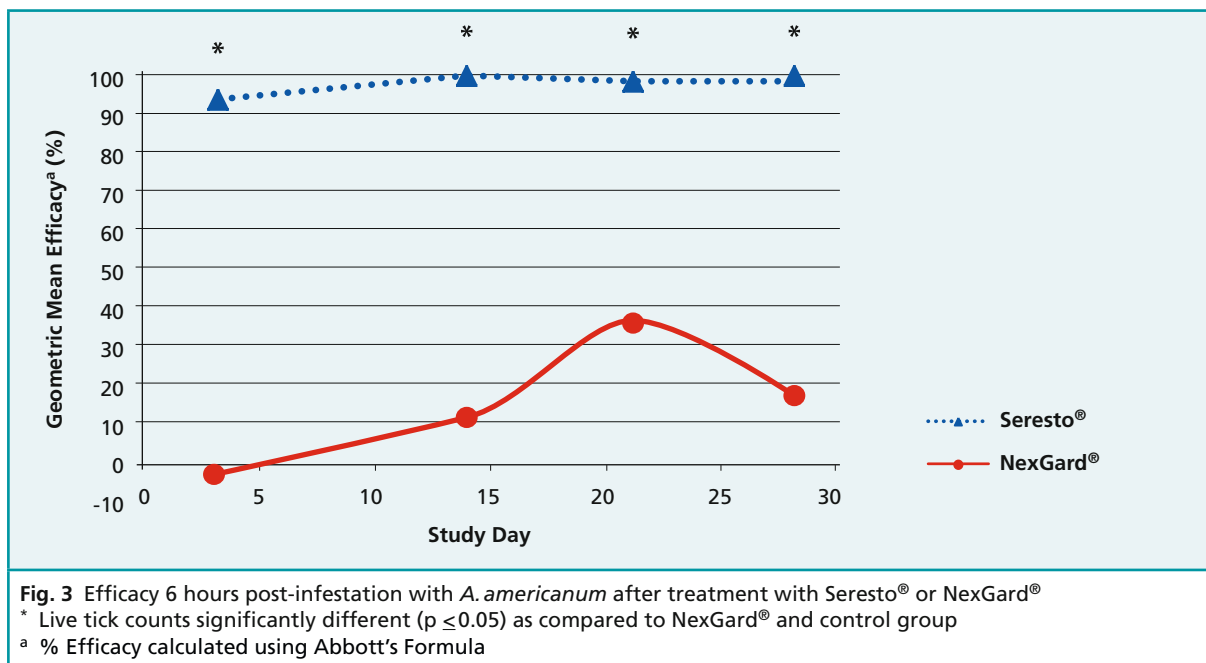


Fig. 2 Efficacy 12 hours post-infestation with *D. variabilis* after treatment with Seresto® or NexGard®
 * Live tick counts significantly different ($p \leq 0.05$) as compared to NexGard® and control group
^a % Efficacy calculated using Abbott's Formula

Results

Thirty dogs (16 females and 14 males) were included in Groups 1, 2 and 3. All dogs were over 6 months of age and ranged in weight from 6.5–15.3kg. Dogs were dosed according to the label

directions. In Group 1, there was 1 dog treated with the small dog Seresto® collar (< 18 lbs) and 9 dogs were treated with the large dog collar (> 18 lbs). In Group 2, the dose ranged from 3.1–6.2mg/kg. There were no adverse events during the study. Efficacy against *D. variabilis* at 6 hours for Group 1



was 95–100% and for Group 2 was 38–48% (Table 1, Fig. 1); efficacy at 12 hours for Group 1 was 97–100% and for Group 2 was 27–59% (Table 1; Fig. 2). Efficacy against *A. americanum* at 6 hours for Group 1 was 94–100% and for Group 2 was <0–38% (Table 2; Fig. 3); efficacy

at 12 hours for Group 1 was 98–100% and for Group 2 was 1–40% (Table 2; Fig. 4). The majority (average >87% for both species) of live ticks on the NexGard treated dogs at 6 hours post-infestation were attached to the dogs and not loose on the body; at 12 hours, an average of >99% of live ticks

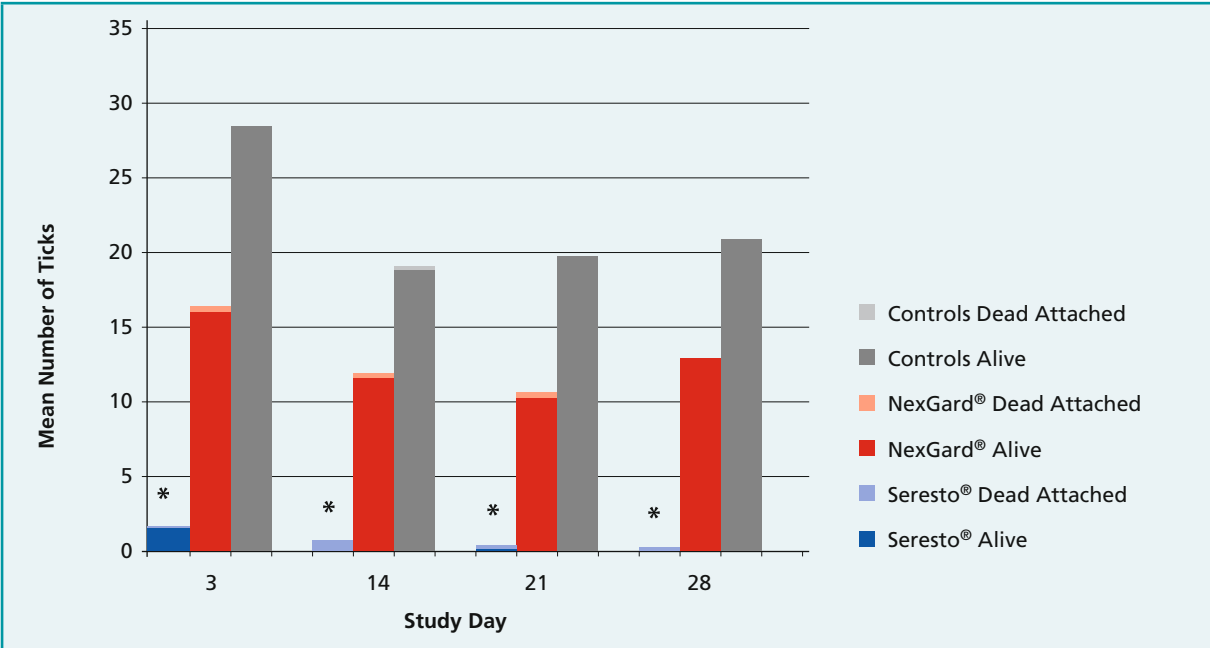


Fig. 5 *Dermacentor variabilis* 6 hour geometric mean live (attached and non-attached) and dead attached tick counts after treatment with Seresto® or NexGard®

*Total tick counts significantly different ($p \leq 0.05$) as compared to NexGard® and control group

Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

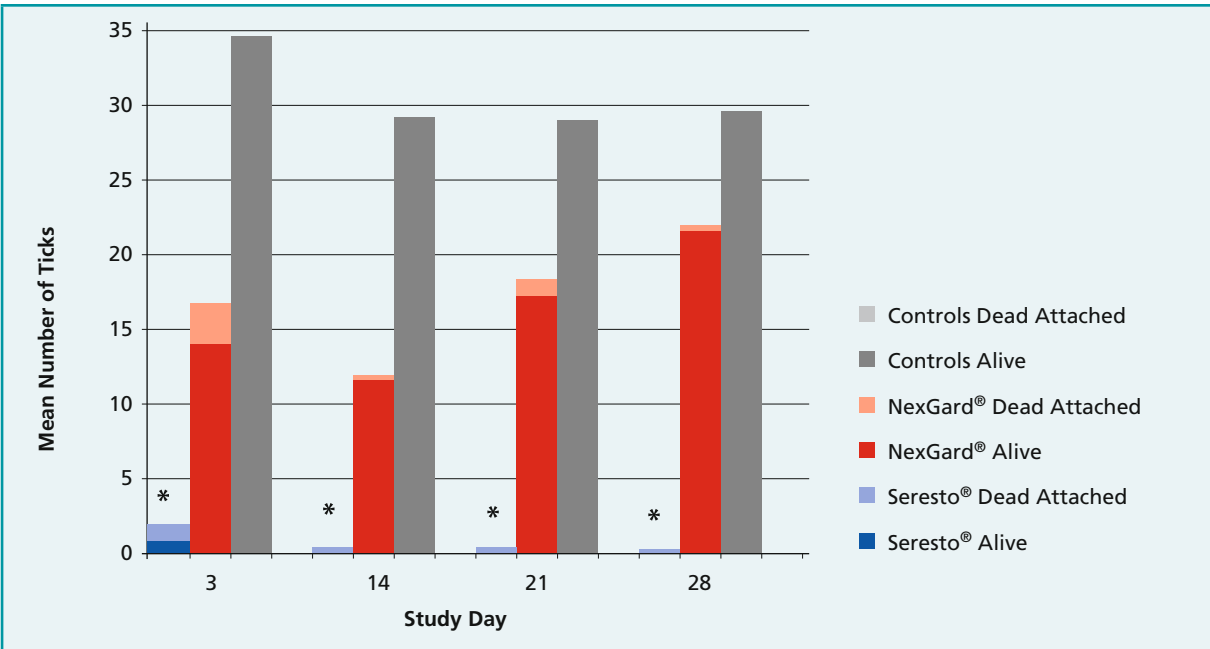


Fig. 6 *Dermacentor variabilis* 12 hour geometric mean live (attached and non-attached) and dead attached tick counts after treatment with Seresto® or NexGard®

*Total tick counts significantly different ($p \leq 0.05$) as compared to NexGard® and control group

Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

on NexGard dogs were attached and feeding. Live (attached and non-attached) and total (total live and dead attached) tick counts in Group 1 against both tick species were significantly lower ($p \leq 0.05$) than Group 2 at both 6 and 12 hours post-infestation on all challenge days. Also, there were significantly fewer ($p \leq 0.05$) live and total ticks of both species on Group 1 dogs compared to Group 3 (control) dogs at all time points (Table 1–2, Fig. 5–8); the number of live or total ticks on Group 2 dogs were never significantly lower when compared to the respective number of ticks on Group 3 dogs. When the two species were combined (sum total of average of *D. variabilis* and *A. americanum*), total tick counts in Group 1 were at most only 3.0 ticks/dog, whereas Group 2 reached 37.9 ticks/dog, and Group 3 reached 52.4 ticks/dog.

Discussion

Ticks are prevalent throughout the world, transmit more pathogenic organisms than any other arthropod, and compete with mosquitoes as being the most important vector of diseases affecting dogs, livestock, and humans (Dantas-Torres et al. 2012; Jongejan and Uilenberg 2004). The incidence of tick-borne diseases in humans and animals is increasing worldwide as the spread of ticks into new areas continues and enhanced molecular biology techniques have identified new species, strains, and genetic variations of microorganisms further increasing the number of tick-borne diseases (Duh et al. 2010; Gray et al. 2009; Jongejan and Uilenberg 2004; Nicholson et al. 2010; Pacheco et al. 2011; Paddock and Childs 2003; Savage et al. 2013). A study published in 2014, which evaluated dogs in the United States, reported a mean prevalence of 7.2% (509,195/6,996,197) for *B. burgdorferi*, 1.6% (111,673/6,994,683) for *E. canis*, and 4.4% (270,168/6,192,268) for *Anaplasma phagocytophilum* (Little et al. 2014). In comparison to a previous report in 2009, the prevalence of dogs with antibodies to *B. burgdorferi* and *E. canis* increased

from 5.1% and 0.6% respectively; the prevalence of *A. phagocytophilum* decreased slightly from 4.8% (Bowman et al. 2009).

Due to the potentially severe clinical implications of fleas, ticks, and the pathogens they can vector, products should be administered to dogs and cats year-round (Baneth et al. 2012; CAPC 2011). Furthermore, to aid in the prevention of pathogen transmission, a product should ideally prevent ticks from attaching and feeding (Spencer et al. 2003). If repellency is not possible, a product must exert its anti-feeding/kill effects quickly, before transmission can occur (Blagburn et al. 2004; Spencer et al. 2003). Guidelines from the 2011 Canine Vector-Borne Disease (CVBD) world forum recommend prevention as the best approach to inhibit transmission of disease causing organisms with 3 specific strategies (Baneth et al. 2012). First, as pathogen transmission can occur almost immediately from most vectors and some ticks, a product with repellent properties should be considered to avoid blood feeding by arthropods. Second, animals should be protected from as many parasites as possible. Finally, year-round flea and tick control should be maintained. In 2006, a Lyme disease consensus statement by the American College of Veterinary Internal Medicine (ACVIM) further emphasized the importance of repellency, especially for rapidly transmitted diseases, and stated that “any of these [permethrin/imidacloprid, fipronil, amitraz collar, and other permethrin-containing products] products can be effective in reducing transmission of Bb [*B. burgdorferi*] to dogs. However, products that prevent tick attachment or repel ticks are needed to decrease transmission of other tick-borne infections” (Littman et al. 2006).

Seresto® is a uniquely formulated collar and one of the few products available that not only kills but also repels fleas and ticks on dogs and cats. Recent research supports the conclusions by the CVBD forum and the ACVIM consensus statement that products with repellent properties are important to aid in the prevention of vector-borne disease transmission. Specifically, Seresto® has been shown to

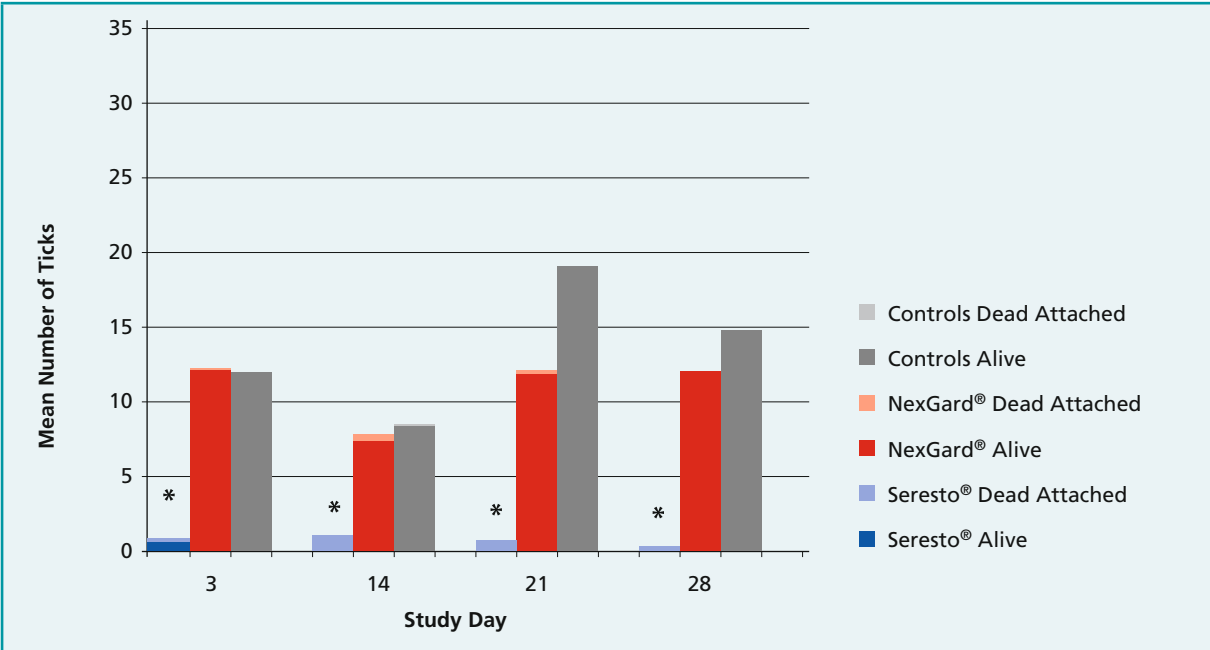


Fig. 7 *Amblyomma americanum* 6 hour geometric mean live (attached and non-attached) and dead attached tick counts after treatment with Seresto® or NexGard®
*Total tick counts significantly different ($p \leq 0.05$) as compared to NexGard® and control group
Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

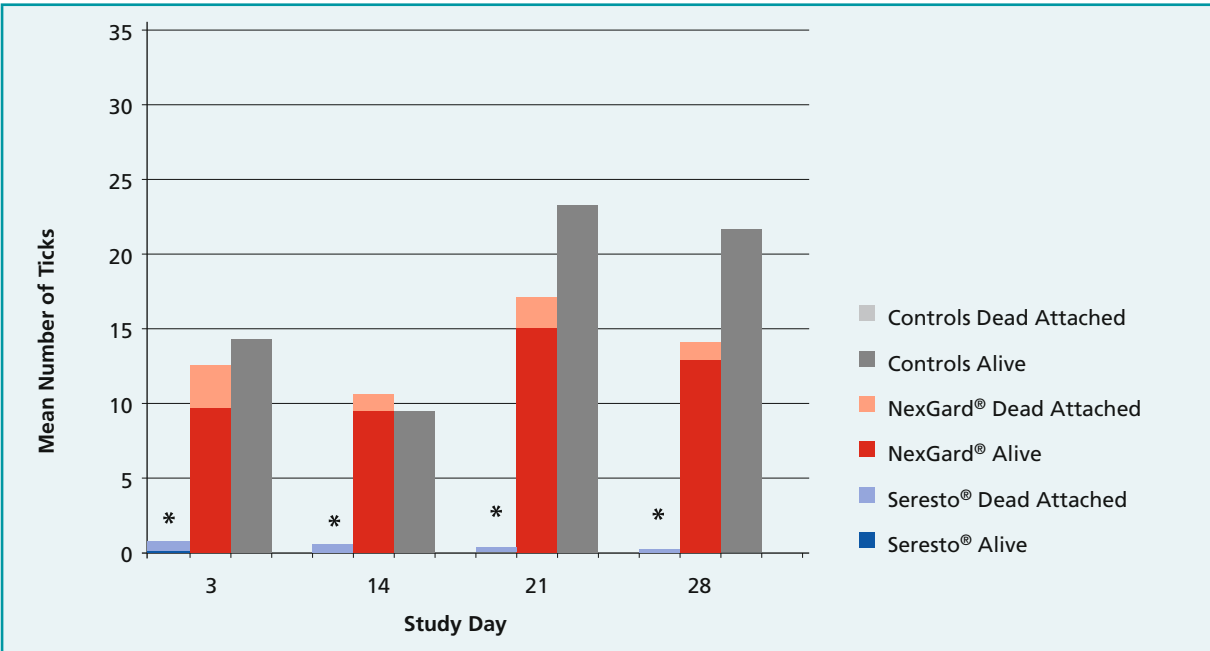


Fig. 8 *Amblyomma americanum* 12 hour geometric mean live (attached and non-attached) and dead attached tick counts after treatment with Seresto® or NexGard®
*Total tick counts significantly different ($p \leq 0.05$) as compared to NexGard® and control group
Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

aid in the prevention of *E. canis*, *Babesia canis*, *Babesia vogeli*, and *Anaplasma platys* transmission in dogs and *Cytauxzoon felis* transmission in cats (Dantas-Torres et al. 2013; Fourie et al. 2013a; Reichard et al. 2013; Stanneck et al. 2012). Seresto® also aided in the prevention of *Bartonella henselae* transmission by *C. felis* and *Leishmania infantum* transmission by sand flies (Brianti et al. 2014; Lappin et al. 2013; Otranto et al. 2013).

In this study, Seresto® was highly efficacious (94–100%) at repelling and killing *D. variabilis* and *A. americanum* ticks as soon as 6 hours post-infestation starting 3 days after application. The results illustrate the rapid repellent properties of the product by inhibiting almost all ticks from attaching and feeding within 6 hours of infestation. Ticks that are repelled and killed are unable to attach and feed, therefore decreasing the risk of disease causing organism transmission, as exemplified in the aforementioned studies. In the authors' clinical experience, these same repellent properties are observed after placing ticks on Seresto® treated dogs. Ticks appear uncomfortable and irritated almost immediately, crawling aimlessly on the haircoat until they fall off of the dog and are killed. This irritant action is further characterised in the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Marchiondo et al. 2013). Sensus stricto repellency is characterised, according to the WAAVP, by ticks that are found either moving away from the treated animal or that fall off within 6–8 hours after contact with treated haircoat, both characteristics of ticks applied to Seresto® treated dogs.

In contrast to Seresto®, the number of ticks on NexGard® treated dogs was never statistically different from non-treated control dogs throughout the study. Out of the 50 ticks of each species applied to the dogs, the geometric mean live tick counts on NexGard® treated dogs were up to 21.6 *D. variabilis* and 15.1 *A. americanum* ticks. This is in comparison to Seresto® treated dogs with up to 1.5 live *D. variabilis* and 0.7 live *A. americanum* ticks. Even at 12 hours, most of the ticks attached to the NexGard®

treated dogs were still alive and feeding. If infected, these ticks would have the potential to transmit pathogens. Similar efficacy results for NexGard® were reported in dogs infested with *Ixodes ricinus* ticks, a species not found in North America (Halos et al. 2014). In that study, the 12 hour efficacy the day of treatment was high (93.4%) but rapidly declined over the month to 76.6%, 41.9%, 36.9%, and 38.5% on days 7, 14, 21, and 28 respectively. It should be noted that the WAAVP guidelines suggest that tick efficacy be determined 48 hours after an infestation and in rare cases, efficacy may be determined at 72 hours (systemic products or when taking into account the potential transmission of pathogens) (Marchiondo et al. 2013). Therefore, despite the fact that pathogens can be transmitted within these long time periods, the purpose of this study was not to discredit the actual label claims for NexGard®. The purpose was to determine the speed of repellency and/or kill of both products. Further studies should be performed to determine the effect of NexGard® treatment on preventing transmission of disease causing organisms.

Conclusion

In this study, Seresto® (imidacloprid/flumethrin collar) was highly efficacious (94–100%) at killing *D. variabilis* and *A. americanum* ticks on dogs at 6 and 12 hours post-infestation and was significantly more efficacious ($p \leq 0.05$ based on live tick counts) than NexGard® (afoxolaner) on all challenge days. The efficacy of NexGard® was never significantly different from non-treated control dogs ($p > 0.05$ based on live tick counts). There were also significantly fewer total ticks (sum of live attached, live non-attached, and dead attached) of each species ($p \leq 0.05$) on dogs treated with Seresto® compared to dogs treated with NexGard® at all time points. The results of this study support the rapid repel and kill properties of Seresto® that are important to aid in preventing transmission of disease causing organisms.

Ethical standards

The study was performed in compliance with current national laws and regulations.

Funding

The study was funded by Bayer HealthCare Animal Health.

Conflict of interest

Cameon M. Ohmes, Joe Hostetler, Wendell Davis and Terry Settje are employed by Bayer Health Care Animal Health. Terry Settje performed the statistical analyses. William Russell Everett is a consultant for Bayer Health Care Animal Health.

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